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Fall webworm host plant preferences create enemy-free space in its interaction with parasitoids

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Abstract

Based on the "enemy-free space" hypothesis, in order to avoid natural enemies, insects may prefer host plants that are nutritionally suboptimal but are less visited by their natural enemies. The fall webworm, Hyphantria cunea Drury has more than 600 reported hosts. Chouioia cunea, a parasitoid wasp, is the main natural enemy of H. cunea. We addressed the question whether the preference of H. cunea for host plants correlate with attractiveness of the plants to C. cunea. H. cunea larvae were reared on leaves of eight different host plants, and the relationship between H. cunea host preference and preferences of the parasitic wasp were evaluated. The preferred host plant of female H. cunea was mulberry, Morus alba. Compared with other plants, M. alba was a poor nutritional host for H. cunea. However, compared with other host plants, M. alba attracted fewer natural enemies C. cunea. GC-MS combined with GC-EAD analysis revealed six compounds in the volatiles of different plant HIPVS after feeding by H. cunea. Behavioral assays showed that tridecane alone did not elicit any attraction or repellency responses of the host H. cunea or the parasitoid C. cunea. When tridecane was mixed with other HIPVS, however, the mixture could attracted C. cunea and repelled H. cunea. Notably, only M. alba HIPVS did not contain tridecane. Thus, H. cunea exploits M. alba as an enemy-free space, minimizing attacks by the parasitoid C. cunea. This information underscores that adaptive responses of herbivores need to be considered in the context of multi-trophic relationships rather than optimizing herbivore growth on the most nutritionally adaptive plant host.

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Fall webworm host plant preferences create enemy-free space in its interaction with parasitoids

Lina Pan[1#], Wenfang Gao[1#], Xinyu Liu[1], Dongyu Qin[1], Jiayi Han[1], Yukun Wei[1], Tiantian Zhang[1], Rui Ren[1], Zeyang Sun[1], Penghua Bai[2], Jing Wang[3], Yonghong Yao[4], Pengfei Hu[5], Min Li[1*]

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Statement of authorship:

Min Li and Lina Pan designed the study, Wenfang Gao and Xinyu Liu, Dongyu Qin, Jiayi Han, Yukun Wei, Tiantian Zhang, Rui Ren, Zeyang Sun performed the research, Penghua Bai, Jing Wang, Yonghong Yao, Pengfei Hu provided new methods or materials, and Lina Pan wrote the manuscript.

Data Availability Statement

Authors have data permissions for all data used in this study. Data deriving from published sources are referenced in the manuscript. The datasets used in this study will be available from the Tianjin Normal Unversity, college of life sciences data center.

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Abstract

Hyphantria cunea Drury has more than 600 reported hosts.*Chouioia cunea* is the main natural enemy of *H. cunea* . We addressed the question whether the preference of *H. cunea* for host plants correlate with attractiveness of the plants to *C. cunea* . *H. cunea* larvae were reared on leaves of different plants. The preferred plant of female *H. cunea* was *Morus alba.* Compared with other plants, *M. alba* was a poor nutritional host for *H. cunea.* However, compared with other plants, *M. alba* attracted fewer natural enemies. GC-MS combined with GC-EAD analysis revealed six compounds in the volatiles of different plant HIPVS. Behavioral assays showed that when tridecane was mixed with other HIPVS, the mixture could attracted *C. cunea* and repelled *H. cunea* . Notably, only *M. alba* HIPVS did not contain tridecane. This information underscores that adaptive responses of herbivores need to be considered in the context of multi-trophic relationships.

Key words: enemy-free space, tritrophic interactions, parasitoid,*Hyphantria cunea, Chouioia cunea,* GC-EAD, tridecane

Introduction

There are many physical, physiological and behavioral mechanisms that can result in the emergence or support of 'enemy-free space' (Biere et al. 2002; Singer et al. 2004; Hu et al. 2020). For example, the gall wasps defence against natural enemies by rapid changing in gall morphology may allow to attain a measure of 'enemy-free space' (Jeffries & Lawton 1984; Price et al. 1987; Singer & Stireman 2003). Herbivores may evolve behavioral preferences to eat or oviposit on host plants that are nutritionally suboptimal, but are less visited by their natural enemies. Overall, herbivores preferring these less optimal plant species may experience better survival than on more nutritious hosts on which the herbivore is more vulnerable to parasitization (Ohsaki & Sato 1994; Lill et al. 2002; Mulatu et al. 2004; Singer et al. 2004; Alhmedi et al. 2021). Studies suggest that the strong influence of host plants on the risk of attack by parasitoids is a potentially important selective force in the evolution of herbivore diet breadth (Vidal & Murphy 2018). For example, larvae of the generalist arctiid moth *Platyprepia virginalis* experienced 83% parasitism by tachinid flies on hemlock (*Conium maculatum*), whereas only 50% of the caterpillars collected from lupin (*Lupinus albus*) in the same habitat were parasitized (Englishloeb et al. 1993). If the heavy toll taken by the parasitoid on hemlock is not compensated by some physiological or ecological advantage, the insect may be expected to evolve an avoidance reaction to this host, which, although nutritionally equivalent to lupin, is suboptimal in terms of risk of parasitization. Another example of a generalist herbivore feeding on physiologically suboptimal plants and thus reducing its mortality rate after parasitization is the arctiid *Grammia geneura* . Its larvae feed preferentially on a several plant species, and were found to include in their diet certain plant species that are nutritionally inferior, but provide chemicals that increase their chances of survival after parasitization (Singer & Stireman 2003). From these and many other examples (Williams et

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al. 2001; Singer et al. 2004; Murphy et al. 2014; Hu et al. 2020), we may infer that host-plant preferences are governed not only by nutritional quality, but also by the third trophic level, natural enemies.

The fall webworm, *Hyphantria cunea* Drury is a worldwide pest. Since it was first introduced into China in 1979, the moth has invaded provinces and cities, including Liaoning, Shandong, Anhui, Shanxi, Hebei, Henan, Shanghai, Tianjin and Beijing, and it continues to spread (Ji et al. 2003; Gao et al. 2010). The larvae have more than 600 reported host plants, about 100 of which occur in China (Zhang & Wang 2009). The parasitoid wasp *Chouioia cunea* Yang (Hymenoptera: Eulophidae) (Yang 1989) is an endoparasitic chalcid wasp, native to China, that parasitizes the fall webworm. A total of 145–365 adult wasps may emerge from a single *H. cunea* pupa and the percentage of emerged female wasps was very high (98–99%) (Yang 1995). In China,*C. cunea* is the main natural enemy of the *H. cunea* and it has shown great promise for reducing *H. cunea* populations in a biological control program (Yang 2004; Yang et al. 2008; Zheng et al. 2012; Zhu et al. 2017).

Although *H. cunea* can survive on hundreds of plants, larvae prefer certain host plants to others (Li et al. 2018; Hu et al. 2019; Wang et al. 2020). It is unknown whether the nutritional optimal plants for *H. cunea* are also the most preferred hosts for *H. cunea* . Moreover, it is unknown preferred host plants are also most attractive to both *H. cunea* and its parasitoid *C. cunea* . In this paper, we reared *H. cunea* larvae on leaves of eight different host plants until pupation. Performance of *H. cunea*larvae on different host plants was assessed by their growth rate. We also assessed the relative orientation preferences of mated *H. cunea* females for the eight different plants. Independently, we compared the attraction of *C. cunea* to different plants before and after they were fed upon by *H. cunea* larvae. Finally, using GC-MS and GC-EAD, we identified the biologically active components of the volatiles of the different plants before and after feeding by*H. cunea* , and evaluated the effects of these compounds on*C. cunea* and *H. cunea* in behavioral assays.

Infochemicals provide important cues for insects to locate and recognize their hosts and avoid enemies (Costa & Reeve 2011; Lo Giudice et al. 2011; Mouratidis et al. 2021). However, most of the current research has rarely analyzed the relationships among plants, insects and natural enemies from the point of infochemical communication. In this study, the chemical ecology and behavior of both the herbivore and the parasitoid were integrated, which could be an important key to solving the mystery of 'enemy free space'.

Material and Methods

2.1 Insects rearing

Parasitoid wasps *C. cunea* was obtained from the Natural Enemy Breeding Center of Luohe Central South Forestry (Henan, China) in 2012. The tussah, *Antheraea pernyi* (Lepidoptera: Saturniidae), was the substitute host of *C. cunea* , and these were obtained from the Benxi Tussah Breeding Base (Liaoning, China). The cultivation of*C. cunea* see Zhu et al., 2017. *H. cunea* eggs obtained from the Chinese Academy of Forestry in 2020 (where they were reared on artificial diet), were allowed to hatch and the larvae were raised in the laboratory in on leaves of various species, including mulberry (*Morus alba* L.), peach (*Amygdalus persica* L. var. persica f. duplex Rehd), empress tree (*Paulownia tomentosa*), tree of heaven (*Ailanthus altissima* (Mill.) Swingle), weeping willow (*Salix babylonica*), Korean aspen (*Populus davidiana*), Chinese ash (*Fraxinus chinensis*), and Old World sycamore (*Platanus orientalis* Linn.).

2.2 Performance of larvae on eight host plants

Three sets of 100 hatchlings were set on each plant species. As the body length and weight of the *H. cunea* varied very little before the fourth instar, so when the larvae reached the fourth instar, 10 larvae were randomly selected from each cage every two days and their body length and body weight were measured. The pupation rate was calculated after all the larvae pupated. The cages were kept in an incubator at 25°C and 70% relative humidity (RH) with 14:10 light: dark cycle.

2.2 Collection of volatiles

The leaves of eight plant species were collected at the Tianjin Normal University campus on the day of the

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experiment. They were soaked in water for two hours to remove biologicals and pesticides and then air-dried naturally. The leaves were divided into two groups for dynamic volatile collections. The first group was not exposed to herbivores before headspace collection. The second group was exposed to 5th instar larvae of *H. cunea* that were starved for 12 hrs. After 6 hrs of feeding on the leaves, the larvae and the frass were removed, and the headspace collection was started.

Twenty g of plant leaves were placed in each 1-L glass conical flasks. Room air was pumped through activated charcoal into the conical flask at a flow rate of 600 mL-min^{-1}. A glass tube (8.5x0.35cm internal diameter) containing Porapak Q (100 mg) (Waters, Shanghai, China) sandwiched between glass wool plugs was placed at the downwind outlet. Air was drawn from the chamber through the Porapak tube at a flow rate of 500 mL-min^{-1}. Thus, positive pressure within the chamber ensured that no room air leaked into the chamber. Volatile compounds were collected in the Porapak Q traps for 6 h and eluted with 1 mL of freshly distilled hexane. The sample was concentrated to 100 µL under a gentle stream of nitrogen and was stored at -20$^{[?]}$C until further use.

2.3 Gas chromatography and GC-MS

The collected volatiles were analyzed by Agilent GC 7890A equipped with a 30 m × 250 µm × 0.25 µm DB-5MS column (Agilent Technologies, Santa Clara, California, US) with splitless injection (250$^{[?]}$C). The oven temperature was maintained at 50$^{[?]}$C for 1 min, then programmed at 4$^{[?]}$C min^{-1} to 150$^{[?]}$C and subsequently held at this temperature for 1 min, then programmed at 10$^{[?]}$C min^{-1} to 230$^{[?]}$C followed by a 10 min hold. The carrier gas was helium (1.2 mL-min^{-1}).

Electrophysiologically active GC peaks were analyzed using an Agilent 7200 Q-TOF mass spectrometer (EI, 70eV, source temperature 2 50$^{[?]}$C, m/z 50–650). Tentative identification of the*C. cunea* EAD-active compounds were identified based on Kovats indices, electron ionization mass spectra and comparison with authentic chemicals.

2.4 Gas chromatography-electroantennography

An antenna of a 1-day-old mated *C. cunea* female was cut off at its base using surgical scissors under a magnifying lens. A glass capillary (0.5 mm inner diameter) filled with 0.1 M KCl solution was used as an electrode. The reference electrode was connected to the base of the isolated antenna, and the recording electrode was connected to the cut tip of the antenna. Chlorinated silver–silver chloride junctions were used to maintain electrical contact between the electrodes and input of the preamplifier. The analog signal detected through a probe (INR-II; Syntech) was processed using a data acquisition controller (IDAC-232; Syntech) and was later analyzed using customized software (Syntech). In the coupled GC-EAD, the effluent from the GC column was simultaneously directed to the antennal preparation and GC detector (Flame Ionization Detector, FID). The collected pupal volatiles were separated by GC (6820, Agilent Technologies, Stockport, UK) using a 30 m × 0.32 mm × 0.25 µm ID HP-5MS column (Agilent). The oven temperature was maintained as described above. The carrier gas was helium. The EAD amplifier output and the FID were monitored simultaneously and analyzed using Syntech software. A peak was deemed electrophysiologically active if it elicited responses on three or more antennal preparations.

2.5 Chemicals

Synthetic standards required for the confirmation of identity and behavioral bioassays were purchased from J&K Chemical Co. (Beijing, China) (nonadecane 99.5%, CAS 629-92-5; hexadecane 99%, CAS 544-76-3) and Aladdin Co. (Shanghai, China) (Undecane [?]99%, CAS 1120-21-4; heneicosane >99%, CAS 629-94-7; tridecane 99%, CAS 629-50-5; tetradecane 99%, CAS 629-59-4).

2.6 Behavioral assays

2.6.1 Responses of mated female *H. cunea* to different plants

Because feeding on different plant leaves might affect their later selection behavior, we fed *H. cunea* an artificial diet. Single leaves of approximately equal weight (5 g) of eight plant species were cleaned by water

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and natural air-dried, and two leaves were fixed to each side of a cage (30 x 30 x 30 cm) with adhesive tape. The experiment was carried out in a dark room. Fifteen mated female moths were placed in three petri dishes, which were put in the center of the cage. The petri dishes were uncovered and moth behavior was observed for 30 min. For each of eight replications we used new plant leaves and new *H. cunea* females.

2.6.2 Responses of 1-day-old mated *C. cunea* females to various odor sources

Y-tube (binary choice) and four-arm olfactometers were used to assess the behavioral responses of 1-day-old mated *C. cunea* females to various chemicals. The Y-tube olfactometer consisted of a common glass tube (11.5 cm long x 2.2 cm diameter) and two lateral glass arms (7.5 cm long x 2.2 cm diameter). Air (200 mL min^{-1}) was delivered from an air pump through activated charcoal and doubly distilled deionized water to humidify the air. The experiment was conducted in a dark room. A lamp (25W, 250 lx) was positioned 55 cm above the olfactometer for illumination.

The four-arm olfactometer (QT-WII01, Channel Technology Co., Beijing, China) consisted of an acrylic chamber (10×10 cm) that was divided into four zones. The top of the chamber was covered with a glass plate to prevent insects from escaping. The experiment was conducted in a dark room. A light bulb was positioned on the top of the arena such that the amount to flight(250 lx) was similar for all arms. The experiments involved the following comparisons: (i) different plant leaves before and after they were feed upon by *H. cunea* larvae; (ii) the eight plant leaves after they were fed upon by *H. cunea* larvae; (iii) single compounds at different doses vs hexane (carrier solvent); and (iv) different of synthetic blends (see detailed methods below).

(i) 5 g clean plant leaves were placed in one arm of the Y-tube olfactometer, and 5 g leaves from the same plant, fed upon by *H. cunea* , were placed in the other arm. The leaves were separated using a 70 mesh absorbent cotton gauze to prevent physical contact. Forty mated female *C. cunea* (1 day old) were released at the base of the common arm of the Y-tube and observed for a maximum of 5 min. Females that did not make a choice within 5 min were not included in the analysis. Females that moved 1 cm beyond the Y-junction and remained there for at least 10 s were considered to have made a choice. After each assay, the tube was washed with soap, water and hexane and air dried, and its orientation was changed. Nine independent biological tests were repeated for each odor source. Each parasitoid was used only once. The bioassays were conducted at $25^{[?]}$C and 60% relative humidity (RH). Female response percentage was calculated as follows: Rs = 100% x (parasitoids that chose the treatment arm)/(parasitoids that chose the treatment arm + parasitoids that chose the control arm).

(ii) The responses of *C. cunea* to the eight different plant leaves after they were fed upon by *H. cunea* were investigated using a four-arm olfactometer. The experiment was carried out as follows: Group 1: the attraction of four plants to the wasps was compared; Group 2: attraction to the other four plants was compared; Group 3: the most attractive two plants in group 1 were compared with the most attractive two plants in group 2; Group 4: the least attractive two plants in group 1 were compared with group 2 again. Leaves (5 g) fed upon by *H. cunea* were placed inside the chambers of four randomly selected arms of the olfactometer. Forty mated female *C. cunea* (1 day old) were released in the center of the arena and their choice of the four arms was recorded. A choice was defined as entering one of the four arms and remaining there for at least 10 s. The odor sources in the olfactometer were replaced and their positions were changed at every assay. In addition, the entire arena and odor chambers were cleaned with soapy water followed by hexane and then air dried. Each parasitoid was used only once. Nine independent biological tests were run, and the bioassays were conducted at $25^{[?]}$C and 60% RH.

(iii) For chemical standard testing, test compounds at different doses (10, 100, 1,000 and 10,000 ng/μL, 10 μL) were placed on filter papers. After allowing 20 s for the solvent to evaporate, the filter papers were placed in one arm of the Y-tube olfactometer and a similar filter paper with 10 μL of hexane was placed in the other arm (solvent control). The bioassay procedures with *C. cunea* was the same as that presented above.

(iv) The responses of *C. cunea* to 10 μL of synthetic blends of EAD-active compounds were investigated

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using a four-arm olfactometer. Filter papers with synthetic blends of EAD-active compounds (10 ng/uL for each component), synthetic blends (without tridecane) and hexane were placed inside the chambers of three randomly selected arms of the olfactometer. The fourth arm received 10 μL hexane and served as solvent control. The bioassay procedures with *C. cunea* was the same as that presented above.

2.6.3 Responses of female adult *H. cunea* to different compounds

The response of mated *H. cunea* females to different compounds were investigated using a Y tube olfactometer. Group 1: a filter paper with 10 μL tridecane (10 ng/μL) was placed in one arm of the Y-tube olfactometer and hexane (solvent control) placed in the other arm. Group 2: a filter paper with a synthetic blend of EAD-active compounds without tridecane was placed in one arm of the Y-tube olfactometer, and 10 μL of the same synthetic blend with tridecane (10 ng/uL for each component) was placed in the other arm. The bioassay procedures with *C. cunea* was the same as that presented above.

2.7 Statistical analysis

We used SPSS version 16.0 (SPSS, Chicago, IL, USA) statistical software for all data analyses. Data were analyzed using one-way analysis of variance followed by a post hoc Duncan multiple-range test to compare female *C. cunea* responses to the three lepidopteran pupae, with the level of significance set to $\alpha<0.05$. The results are expressed as the mean ± standard deviation. The response percentages of female *C. cunea* in the Y-tube olfactometer were analyzed using $?^2$ tests.

3 Results

3.1 The growth of *H. cunea* fed on different plant leaves

At the beginning, there was no significant differences in the length of larvae feeding on the leaves of different plants. Later, *H. cunea*that fed on the leaves of the *A. altissima* , *F. chinensis*and *P. tomentosa* grew longer and heavier than on *M. alba* ,*P. davidiana* , *P. orientalis* , *A. persica* and*S. babylonica* ($\alpha<0.05$) (Fig. 1). Pupation rate followed a similar pattern, with significantly more *H. cunea* that fed on the leaves of the *A. altissima* , *F. chinensis* and *P. tomentosa* pupating than *H. cunea* that fed on other plant species.

3.2 *H. cunea* preference of eight plant species

The mated female *H. cunea* chose different plants is not consistent with growth parameters from Fig 1. The preference of them are as follows four levels: 1 *M. alba* ; 2, *P. tomentosa* ,*A. altissima* , *F. chinensis* ; 3, *P. orientalis* ,*P. davidiana* ; 4, *S. babylonica* , *A. persica*($\alpha<0.05$) (Fig. 2).

3.3 Responses of *C. cunea* to leaves before and after feeding by

H. cunea

The leaves of all eight plant species were significantly more attractive to *C. cunea* after being fed upon by *H. cunea* than before*H. cunea* feeding. *F. chinensis* F=43.206, α=0; *P. orientalis,* F=48.970, α=0; *A. altissima* F=60.031, α=0; *P. davidiana* , F=21.008, α=0.002; *M. alba* , F=45.717 α=0; *P. tomentosa,* F=85.462, α=0; *A. persica,* F=24.012, α=0.001;*S. babylonica,* F=11.489, α=0.010 (Fig. 3).

3.3 Comparison of responses of *C. cunea* to different plant leaves fed upon by *H. cunea*

The four-arm olfactometer was used to test the selection rate of*C. cunea* for different plant leaves that had been fed upon by*H. cunea* . Four groups were assayed to compare the attraction of*C. cunea* to different plant species (Fig. 4).

Group I: *F. chinensis* (34.21%) and *A. altissima* (32.29%) attracted more *C. cunea* than *P. tomentosa* (21.45%) and*M. alba* (12.05%) (Fig. 4A).

Group II: *P. orientalis* (32.67%) and *P. davidiana*(31.75%) attracted more *C. cunea* than *A. persica*(16.99%) and *S. babylonica* (18.60%) (Fig. 4B).

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Group III. The four most attractive plants in Groups I and II were compared (Fig. 4C). There were no significant differences in the attractiveness of *F. chinensis* , *A. altissima* , *P. orientalis* and *P. davidiana* (α=0.395).

Group IV: The four least attractive plants in Groups I and II were compared. *P. tomentosa* (38.26%) attracted significantly more*C. cunea* than *A. persica* (24.63%) and *S. babylonica* (26.46%), and *M. alba* attracted the fewest *C. cunea* (10.65%) (Fig. 4D).

Therefore, the attractiveness to *C. cunea* of host plants that were fed upon by *H. cunea* may be ranked into four levels.*F. chinensis* , *A. altissima* , *P. orientalis* and*P. davidiana* were most attractive to *C. cunea* , followed by*P. tomentosa* . *A. persica* and *S. babylonica* were significantly less attractive than the higher ranking five plant species, and *M. alba* was the least attractive to *C. cunea* .

3.4 Chemical analysis

The volatiles of eight different plant leaves before and after feeding by *H. cunea* were analyzed by GC-EAD and GC-MS. Six compounds (undecane, tridecane, tetradecane, hexadecane, nonadecane, heneicosane) in the plant leaves volatiles elicited EAD responses in antennae of*C. cunea* females (Supplementary figure 1). Of the eight plants, tridecane was not detected only in *M. alba* (Table 1).

3.5 Olfactometer bioassays

a) Tridecane vs hexane

Since tridecane was not detected only in *M. alba* leaves, the effect of tridecane on the wasp was evaluated with a 2-choice Y-tube olfactometer. Compared with hexane, none of the concentrations of tridecane elicited significant attraction or repellency of the wasp (10 ng/μl: F=0.063, α=0.805; 100 ng/μl: F=0.583, α=0.456; 1,000 ng/μl: F=1.262, α=0.229; 10,000 ng/μl: F=0.03, α=0.958) (Fig. 5).

b) Responses of *C. cunea* to synthetic blends of EAD-active compounds

Using a four-arm olfactometer, the blank arm and the hexane control attracted few wasps, whereas the synthetic blend of hydrocarbons was highly attractive. However, subtraction of tridecane from the blend resulted in a significant decline in attraction to the blend ($\alpha<0.05$) (Fig. 6).

c) Responses of *H. cunea* females to different compounds

Like *C. cunea* females, *H. cunea* females had no significant preference for tridecane over the hexane control (F=0.10, α=0.920) (Fig. 7A). However, while *C. cunea* females a hydrocarbon blend with tridecane, *H. cunea* females significantly preferred the synthetic blend without tridecane (F=209.764, α=0) (Fig. 7B).

Discussion

Based on the 'enemy-free space' hypothesis, insects may prefer host plants that are nutritionally suboptimal but are less visited by their natural enemies. In this study, *H. cunea* larvae were reared on leaves of eight different host plant, *M. alba* , *A. persica* ,*P. tomentosa* , *A. altissima* , *S. babylonica* , *P. davidiana* , *F. chinensis* and *P. orientalis.* Previous findings indicated that *M. alba* , *P. davidiana* , *P. tomentosa* , *A. altissima* , *F. chinensis* and *P. orientalis* were the preferred host plants of *H. cunea* (Liu et al. 2013), whereas *S. babylonica* and *A. persica* were less preferred host plants of *H. cunea* (Qin et al. 2000).

Our study results indicated that body length, body mass and pupation rate of the fall webworm, *H. cunea* , that fed on the leaves of*A. altissima* , *F. chinensis* and *P. tomentosa* were higher than on leaves of *M. alba* , followed by *P. davidiana*and *P. orientalis* , and finally *A. persica* and *S. babylonica* . Our assays showed that *A. altissima* , *F. chinensis* and *P. tomentosa* provided more nutrition for the growth and development of *H. cunea* than *M. alba* and the other plant species.

In contrast to their growth performance on the various plant species,*H. cunea* females were most attracted to *M. alba* , followed by *P. tomentosa* , *A. altissima* , *F. chinensis* ,*P. orientalis* , *P. davidiana* , *S. babylonica* and*A. persica* . Therefore, compared with other plants, female*H. cunea* preferred *M. alba* . (Yu 2016) found

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that *H. cunea* laid more eggs on *M. alba* than on 11 other host plant species, consistent with our preference results.

Overall, it appears that the preferred host plant of female *H. cunea* (*M. alba*) is not its best nutritional host. Conversely, the best nutritional hosts (*A. altissima* , *F. chinensis* and*P. Tomentosa*) are not the most preferred host plants. A plausible driver of this mismatch between behavioral preference and physiological performance is the third trophic level - natural enemies that impose selection pressure on the behavioral preferences.

Herbivore-induced plant volatiles (HIPVs) play key roles in tri-trophic interaction (Hu et al. 2020; Chen et al. 2021). Therefore, we first investigated the HIPVs released by plants in response to *H. cunea*feeding that attract the natural enemy *C. cunea* . All eight plant species showed a significant increase in their attractiveness to*C. cunea* after feeding by *H. cunea* compared to before feeding. Thus, it is clear that after being fed upon by *H. cunea* , plants release HIPVs that attract the natural enemy *C. cunea* .

Next, we compared the abilities of different HIPVs to attract natural enemies. The attraction of *C. cunea* to *H. cunea* -fed plants was greatest to *F. chinensis* , *A. altissima* , *P. orientalis and P. davidiana* followed by *P. tomentosa* , *A. persica* and *S. babylonica* . *M. alba* was the least preferred plant based on *C. cunea* attraction to its HIPVs. These results indicate that *M. alba* , which is the most preferred host of *H. cunea* , has the weakest ability to attract natural enemies. This may explain why *M. alba* is the most preferred host of*H. cunea* , even though it is not its best nutritional host.*A. persica* and *S. babylonica* have a weak ability to attract *C. cunea* , but *H. cunea* still does not prefer to these plant species, possibly because of their poor nutritional value. Therefore, it appears that *H. cunea* engages in a tradeoff between performance and survival, preferring a host plant with suboptimal nutrition and mitigating parasitism on host plant that offer better nutritional value. Thus, the ideal host plant, *M. alba* , represents a balance between optimal larval performance and survivorship.

To identify the biologically active HIPVs, we analyzed the leaf volatiles of eight different plant species before and after *H. cunea* feeding. GC-EAD, followed by GC-MS analysis identified six electrophysiologically active saturated hydrocarbons (undecane, tridecane, tetradecane, hexadecane, nonadecane, heneicosane).However, tridecane was not detected in only one host plant, *M. alba* .

In several studies, tridecane has been shown to be a HIPV that attracts natural enemies. For example, tobacco plants (*Nicotiana tabacum*L.), infested by sap-sucking aphids (*Myzus persicae* Sulzer) produce tridecane and other compounds as HIPVs (Song et al. 2021). The Asian egg parasitoid *Trissolcus japonicas* uses tridecane as the kairomone of the brown marmorated stink bug, *Halyomorpha haly* for short range host location (Malek et al. 2021). And the predaceous minute pirate bug, *Orius insidiosus* (Say) uses the tridecane as kairomone to locate the brown marmorated stink bug (Fraga et al. 2017). Tridecane is also a component of the alarm volatiles of stinkbugs (*Tessaratoma papillosa*), released when stinkbugs are disturbed or irritated (Zhang et al. 2009). Tridecane is also a component of the pygidial gland of larval secretions, playing a role in chemical defense against predators such as ants (Gasch & Vilcinskas 2014).

Our investigation revealed opposite responses to HPIVs by the herbivore (*H. cunea*) and its parasitoid (*C. cunea*). Alone, tridecane, a prominent HPIV in seven of the eight host plants, did not elicit any behavioral responses – either attraction or repellency – from *H. cunea* or *C. cunea* . However, when mixed with other HPIV compounds, the mixture that included tridecane significantly attracted *C. cunea* and repelled *H. cunea* . *M. alba* , the only host plant that did not emit tridecane in its HPIVs, did not attract the wasps and was preferred by the moth over the other seven host plants.

It is generally believed that the natural enemies rely on HIPVs for long-range localization of an appropriate habitat (Turlings & Wäckers 2004), and the host insect's volatiles for short-range location of the host (Vet & Dicke 1992; van Alphen & Jervis 1996). In this study we identified tridecane, hexadecane, nonadecane, and heneicosane as HIPVS that attracted *C. cunea* wasps. Previously, we identified six compounds (1-dodecene, hexadecane, heptadecane, nonadecane, heneicosane and heptacosane) emitted from pupae of three lepidopteran species, including *H. cunea* , that serve as hosts for *C. cunea* (Li et al. 2020). It is particularly

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notable that both behaviorally active blends (from plant hosts and insect hosts) constitute hydrocarbons with similar characteristics. We therefore propose that the semiochemicals released by plants and those released by host insects may be chemically related and thus perhaps represent convergence of tritrophic systems on suites of related compounds. Identifying common information compounds may be a more efficient strategy for *C. cunea* , matching the parasitoid wasp's limited neural capacity (Schoonhoven et al. 2005).

Conclusion

1 In this study, the preferred host plant of female *H. cunea* was*M. alba.* However, compared with other host plants, *M. alba*was not the best nutritional host for *H. cunea.*

2 Compared with other host plants, *M. alba* was the least attractive to the parasitoid *C. cunea* , a natural enemy of*H. cunea* .

3 GC-EAD and GC-MS analyses identified six volatiles as HIPVs of different host plants fed upon by *H. cunea* . These HIPVs elicited EAD responses in the antennae of female *C. cunea.* Tridecane was emitted by seven of eight host plant species, but not by *M. alba* .

4 Tridecane alone did not attract or repel either *C. cunea* or*H. cunea* , but when mixed with other HPIVs, the mixture significantly attracted *C. cunea* and repelled *H. cunea* .

5 *M. alba* represents "enemy-free-space" for *H. cunea* . By preferring *M. alba* , which does not emit tridecane, *H. cunea* effectively avoids parasitism by *C. cunea* , which is highly attracted to HIPVs that contain tridecane.

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Figure legends

Fig. 1. The body length (A) body mass (B) and pupation rate (C) of*H. cunea* larvae fed on eight plant species leaves. Treatments within each date that do not share letters are significantly different as determined by a one-way ANOVA followed by Duncan's multiple-range test ($\alpha<0.05$).

Fig. 2. Mated female *H. cunea* selection on eight host plants. Mean values in a row with super scripts without a common letter are significantly different as determined by a one-way ANOVA followed by Duncan's multiple-range test ($\alpha<0.05$).

Fig. 3. The responses of *C. cunea* to eight plant species leaves before and after *H. cunea* feeding on the leaves in 2-choice Y-tube olfactometer. Clear bars represent the responses of*C. cunea* to intact (unfed) plant leaves, whereas the solid bars represent the responses to leaves after feeding by *H. cunea.* *Indicates a significant difference compared with the respective control (intact) group as analyzed using $?^2$ tests ($\alpha<0.05$).

Fig. 4. The responses of *C. cunea* to leaves of different plant species that had been fed upon by *H. cunea* in four-arm olfactometer. Plants within a group that do not share common letters are significantly different as determined by a one-way ANOVA followed by Duncan's multiple-range test ($\alpha<0.05$).

Fig. 5. The responses of *C. cunea* females to various doses of tridecane vs. hexane control in 2-choice Y-tube olfactometer.

Fig. 6. The responses of *C. cunea* to different synthetic blends of EAD-active compounds in four-arm olfactometer.

Fig. 7. A: The responses of *H. cunea* to hexane control and tridecane in 2-choice Y-tube olfactometer. B: The responses of *H. cunea* to synthetic blends without tridecane and synthetic compounds blends in 2-choice

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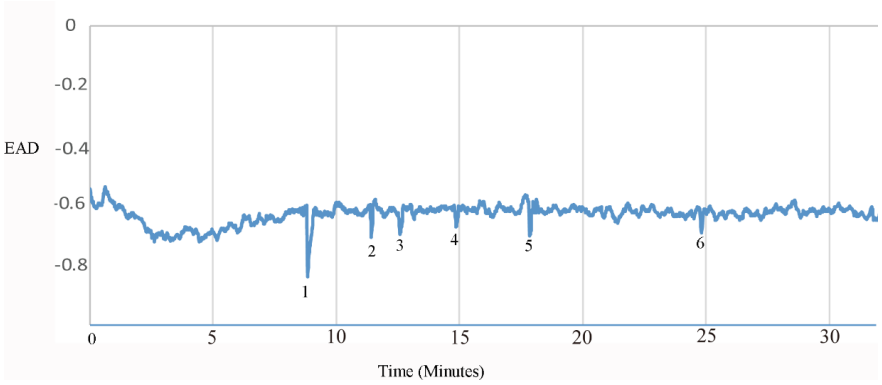
Y-tube olfactometer.

Supplementary figure 1

The mean EAD trace of *C. cunea* to different plants volatiles. The numbers correspond to the identified compounds listed in Table 1.



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Figures

Figure 1

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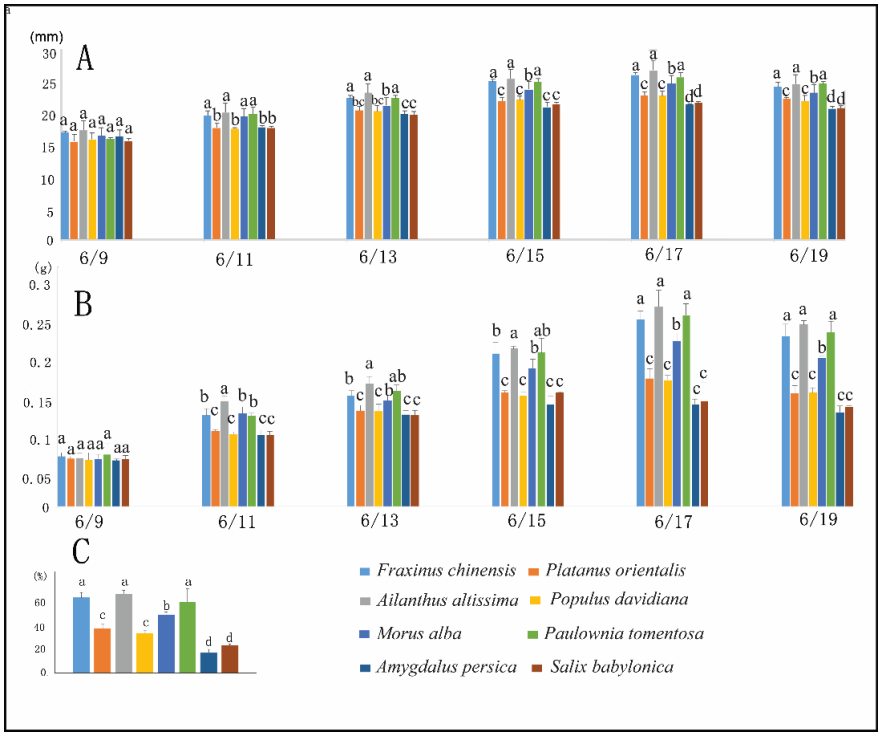

Figure 2

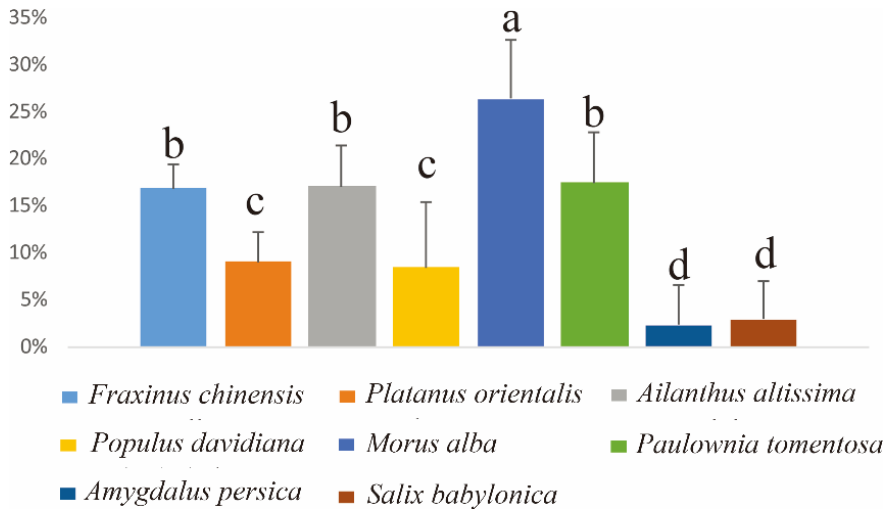

Figure 3

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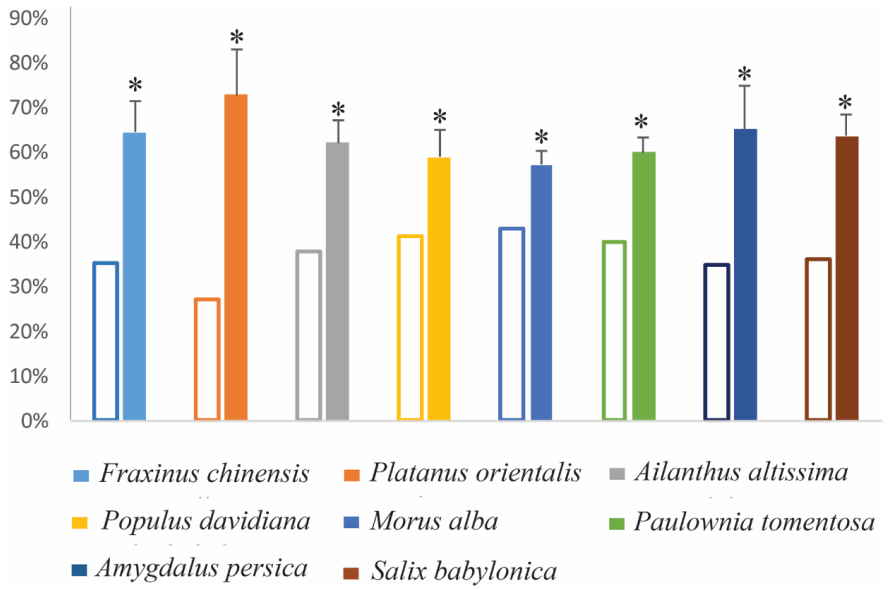

Figure 4

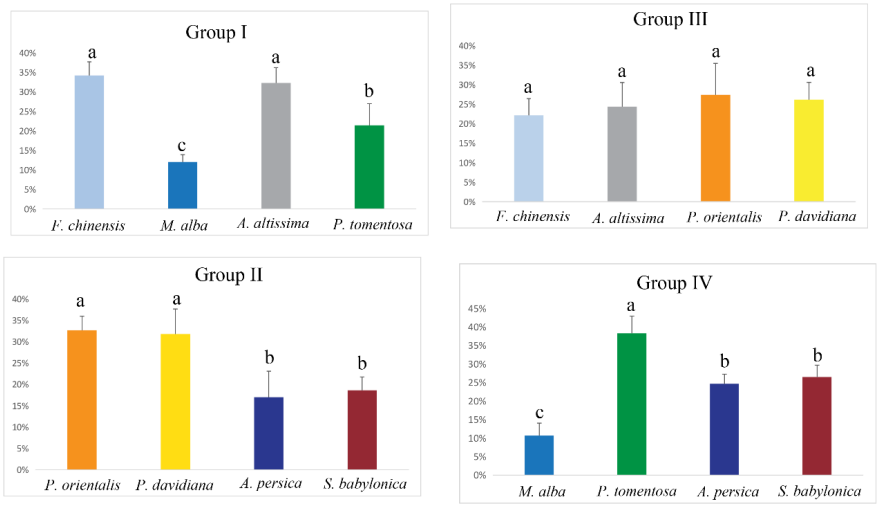

Figure 5

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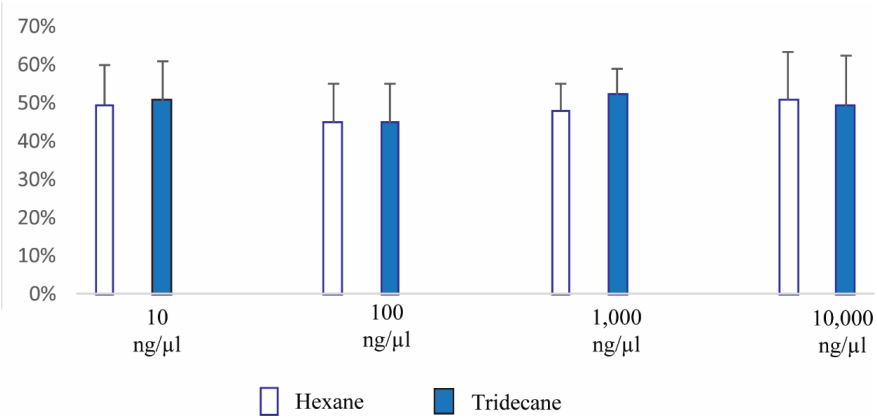

Figure 6

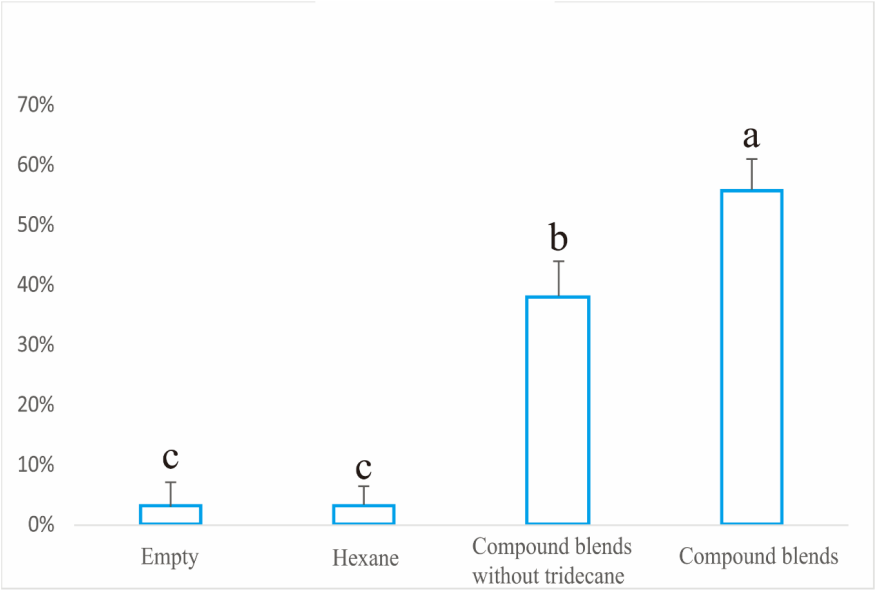

Figure 7

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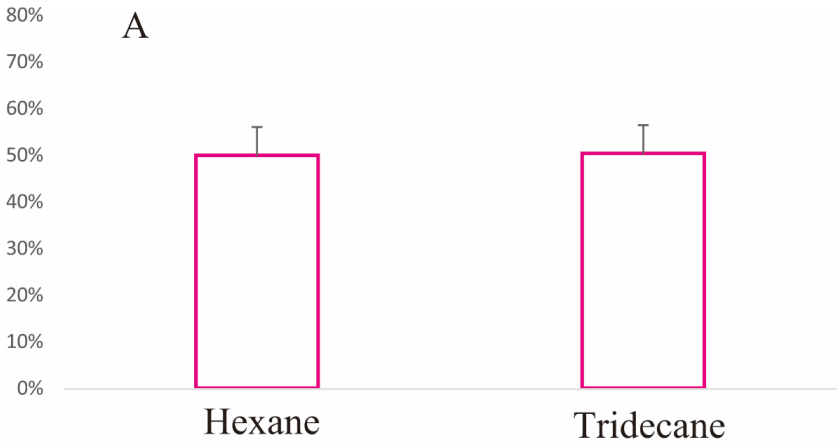

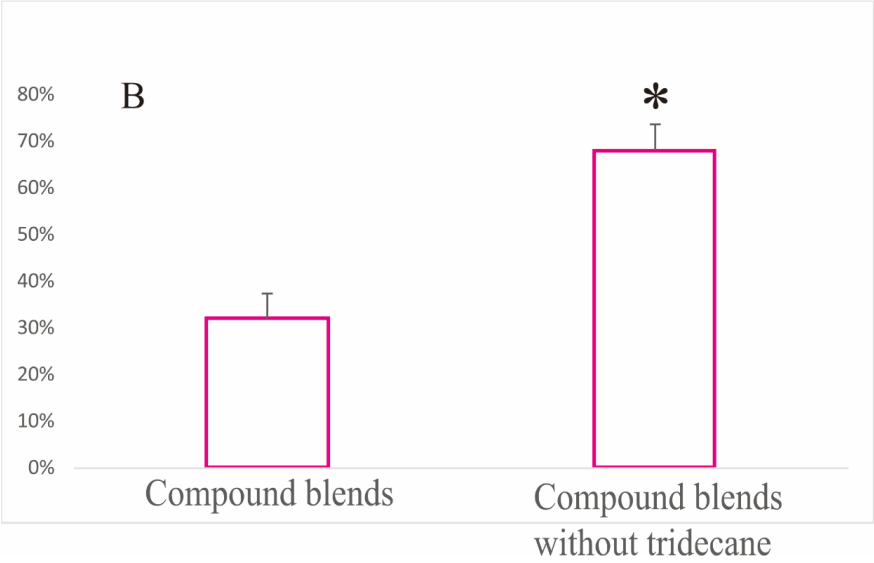

	Compound	Retention time	Emission rate (ng/min from 1 g leaves)	Emission rate (ng/min from 1 g leaves)	Emission rate (ng/min from 1 g leaves)	Emission rate (ng/min from 1 g leaves)	Emission rate (ng/min from 1 g leaves)	Emission rate (ng/min from 1 g leaves)	Emission rate (ng/min from 1 g leaves)
			M. alba	*P. davidiana*	*P. orientalis*	*A. altissima*	*S. babylonica*	*A. persica*	*P. tomentosa*
1	Undecane	8.85	31.48	9.02	17.87	44.36	16.52	15.70	17.51
2	Tridecane	11.43	N	30.76	30.26	30.40	4.98	9.12	21.10
3	Tetradecane	12.60	22.85	15.11	11.41	19.25	13.70	19.91	14.11
4	Hexadecane	14.86	20.63	15.98	23.20	42.31	13.50	21.01	17.21
5	Nonadecane	17.85	17.23	8.91	21.80	24.60	13.68	15.68	13.62
6	Heneicosane	24.81	17.17	11.47	18.57	20.24	13.42	13.95	13.56

Table 1 These compounds were identified by GC peak enhancement using authentic standards. On an Agilent

DB-5 column.

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